

FIG. 1

A. Comparison of disintegrin amino acid sequences:

Contortrostatin	DAPANPCCDAATCKLITGSQCADGLCCDQCKFMKEGTV-C-RARGD ^{DL} -DY-NGISAG-----
Applagin	EAGEECDCGSPENPCCDAATCKLIPGAQCAEGLCCDQCKFMKEGTV-C-RARGDDVNDYCNGISAGCPPRNP ^{FH}
Trigramin	EAGEDCCDCGSPANPCCDAATCKLIPGAQCGEGLCCDQCSFIEEGTVCRIRARGDDLDDYCN ^{GR} SAGCPPRNP ^{FH}
Albolabrin	EAGEDCCDCGSPANPCCDAATCKLIPGAQCGEGLCCDQCSFMKKGTICRRARAGDDLDDYCN ^{GR} SAGCPPRNP ^{LHA}
Elegantin	EAGEECDCGSPENPCCDAATCKLIPGAQCADGLCCDQCKFKKKRTICRRARGDNPDDRCTGOSADCPPR ^{GL} YS
Kistrin	GKECDCSSPENPCCDAATCKLIPGAQCGEGLCCEQCKFDRAGKICRIPRGDMPDDRCTGOSADCPP ^{RYH}

B. Design of PCR primers:

λgt10			
FORWARD		PCR-2	
----->		----->	
	-----DAPANPCCDAATCKLITGSQCADGLCCDQCKFMKEGTVCRARAGDDL-DY-NGISAG-----		
		PCR-1	
		<-----	
			λgt10 REVERSE

C. Overlapping extension of PCR fragments:

(a) self-extendible molecule:

CN-N (approximately 1300 bp)		CN-C (approximately 700 bp)	
5'-----		3'-----	5'
	<---3'		
(b) non-self-extendible molecule:			
CN-N (approximately 1300 bp)		CN-C (approximately 700 bp)	λgt10 reverse
3'-----		5'-----	<-----

----->
λgt10 forward

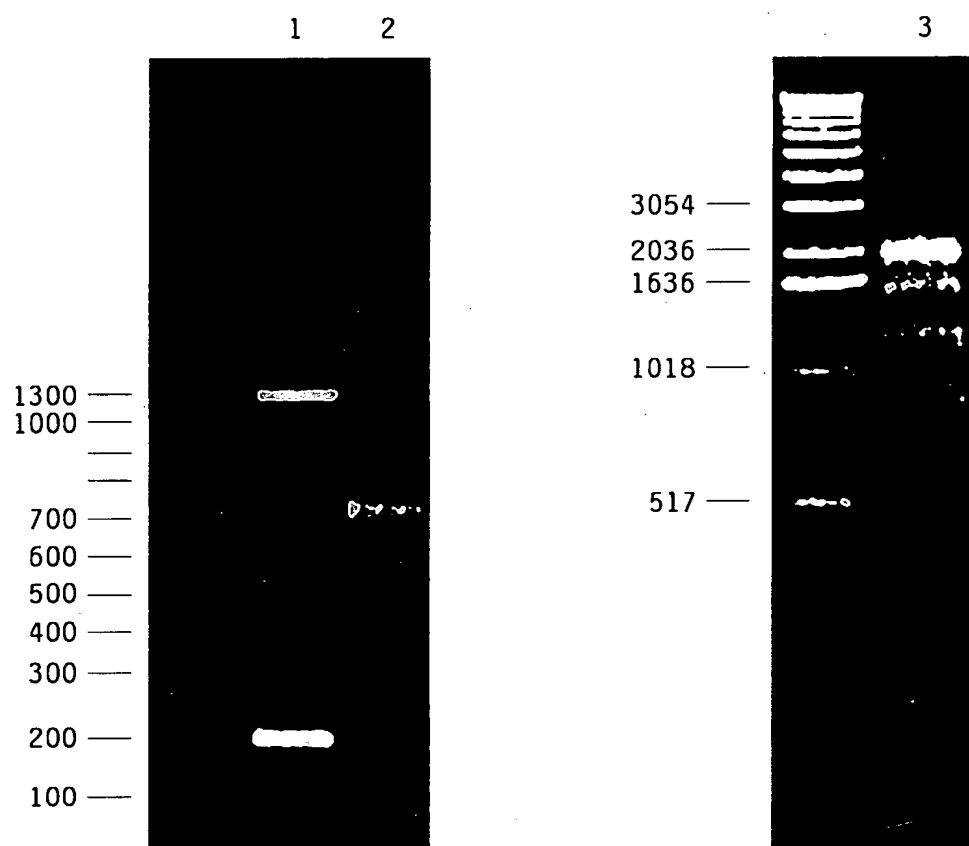


FIG. 2

FIG. 3A

5' GA ATT CGG GGT CAA TAG AGG AAG AGC TCA AGT TGG CTT GAA AGC AGG AAG AGA TTG

11 20 29 38 47 56

65 74 83 92 101 110

CCT GTC TTC CAG CCA AAT CCA GCC GCC AAA ATG ATC CAG GTT CTC TTG GTC ACT

[1] M I Q V L L V T [8]

119 128 137 146 155 164

CTA TGC TTA GCA GCT TTT CCT TAT CAA GGG AGC TCT ATA ATC CTG GAA TCT GGG

[9] L C L A A F P Y Q G S S I I L E S G [26]

173 182 191 200 209 218

AAT GTT AAT GAT TAT GAA GTA CTG TAT CCA CAA AAA GTC ACT GCA TTG CCC AAA

[27] N V N D Y E V L Y P Q K V T A L P K [44]

227 236 245 254 263 272

GGA GCA GTT CAG CCA AAG TAT GAA GAC ACC ATG CAA TAT GAA TTT AAA GTG AAT

[45] G A V Q P K Y E D T M Q Y E F K V N [62]

281 290 299 308 317 326

GGA GAG CCA GTG GTC CTT CAC CTG GAA AAA AAT AAA GGA CTT TTT TCA AAA GAT

[63] G E P V V L H L E K N K G L F S K D [80]

335 344 353 362 371 380

TAC AGC GAG ACT CAT TAT TCC TCT GAT GGC AGA AAA ATT ACA ACA AAC CCT CCG

[81] Y S E T H Y S S D G R K I T T N P P [98]

389 398 407 416 425 434

GTT GAG GAT CAC TGC TAT TAT CAT GGA CGC ATC CAG AAT GAT GCT GAC TCA ACT

[99] V E D H C Y Y H G R I Q N D A D S T [116]

443 452 461 470 479 488

GCA AGC ATC AGT GCA TGC AAC GGT TTG AAA GGA CAT TTC AAG CTT CAA GGG GAG

[117] A S I S A C N G L K G H F K L Q G E [134]

497 506 515 524 533 542

ACG TAC CTT ATT GAA CCC TTG AAG CTT TCC GAC AGT GAA GCC CAT GCA GTC TAC

[135] T Y L I E P L K L S D S E A H A V Y [152]

551 560 569 578 587 596

AAA TAT GAA AAC GTA GAA AAA GAA GAT GAG GCC CCC AAA ATG TGT GGG GTA ACC

[153] K Y E N V E K E D E A P K M C G V T [170]

FIG. 3B

	605	614	623	632	641	650	
	CAG	ACT AAT TGG	GAA TCA GAT	GAG CCC ATC	AAA AAG GCC	TCT CAG TTA	AAT CTT
[171]Q	T	N	W	E	S	D	E P I K K A S Q L N L [188]
	659	668	677	686	695	704	
	ACT CCT GAA CAA CAA	GGA TTC CCC	CAA AGA TAC	ATT GAG CTT	GTT GTA GTT	GCA	
[189]T	P	E	Q	Q	G	F	P Q R Y I E L V V V A [206]
	713	722	731	740	749	758	
	GAT CAC AGA ATG TTC	ACG AAA TAC	AAC GGC AAT	TTA AAT ACT	ATT AGA ATA	TGG	
[207]D	H	R	M	F	T	K	Y N G N L N T I R I W [224]
	767	776	785	794	803	812	
	GTA CAT GAA CTT GTC	AAC ACT ATG	AAT GTG TTT	TAC AGA CCT	TTG AAT ATT	CGT	
[225]V	H	E	L	V	N	T	M N V F Y R P L N I R [242]
	821	830	839	848	857	866	
	GTC TCA CTG ACT GAC	CTA GAA GTT	TGG TCA GAC	CAA GAT TTG	ATC AAC GTG	CAG	
[243]V	S	L	T	D	L	E	V W S D Q D L I N V Q [260]
	875	884	893	902	911	920	
	CCA GCA GCG GCT GAT	ACT TTG GAA	GCA TTT GGA	GAC TGG AGA	GAG ACA GTC	TTG	
[261]P	A	A	A	D	T	L	E A F G D W R E T V L [278]
	929	938	947	956	965	974	
	CTG AAT CGC ATA AGT	CAT GAT AAT	GCT CAG TTA	CTC ACG GCC	ATT GAG CTT	GAT	
[279]L	N	R	I	S	H	D	N A Q L L T A I E L D [296]
	983	992	1001	1010	1019	1028	
	GGA GAA ACT ATA GGA	TTG GCT AAC	AGG GGC ACC	ATG TGC GAC	CCG AAG CTT	TCT	
[297]G	E	T	I	G	L	A	N R G T M C D P K L S [314]
	1037	1046	1055	1064	1073	1082	
	ACA GGA ATT GTT CAG	GAT CAT AGT	GCA ATA AAT	CTT TGG GTT	GCA GTT ACA	ATG	
[315]T	G	I	V	Q	D	H	S A I N L W V A V T M [332]
	1091	1100	1109	1118	1127	1136	
	GCC CAT GAG ATG GGT	CAT AAT CTG	GGT ATT AGT	CAC GAT GGA	AAT CAG TGT	CAT	
[333]A	H	E	M	G	H	N	L G I S H D G N Q C H [350]
	1145	1154	1163	1172	1181	1190	
	TGC GAT GCT AAC TCA	TGC ATT ATG	AGT GAA GAA	CTA AGA GAA	CAA CTT TCC	TTT	
[351]C	D	A	N	S	C	I	M S E E L R E Q L S F [368]

FIG. 3C

	1199		1208		1217		1226		1235		1244							
	GAG	TTC	AGC	GAT	TGT	AGT	CAG	AAT	CAA	TAT	CAG	ACA	TAT	CTT	ACT	GAT	CAT	AAC
[369]E	F	S	D	C	S	Q	N	Q	Y	Q	T	Y	L	T	D	H	N	[386]
	1253		1262		1271		1280		1289		1298							
	CCA	CAA	TGC	ATG	CTC	AAT	GAA	CCC	TTG	AGA	ACA	GAT	ATT	GTT	TCA	ACT	CCA	GTT
[387]P	Q	C	M	L	N	E	P	L	R	T	D	I	V	S	T	P	V	[404]
	1307		1316		1325		1334		1343		1352							
	TCT	GGA	AAT	GAA	CTT	TTG	GAG	ACG	GGA	GAA	AGT	GAC	TTT	GAC	GCT	CCT	GCA	
[405]S	G	N	E	L	L	E	T	G	E	E	S	D	F	D	A	P	A	[422]
	1361		1370		1379		1388		1397		1406							
	AAT	CCG	TGC	TGC	GAT	GCT	GCA	ACA	TGT	AAA	CTG	ACA	ACA	GGG	TCA	CAG	TGT	GCA
[423]N	P	C	C	D	A	A	T	C	K	L	T	T	G	S	Q	C	A	[440]
	1415		1424		1433		1442		1451		1460							
	GAT	GGA	CTG	TGT	TGT	GAC	CAG	TGC	AAA	TTT	ATG	AAA	GAA	GGA	ACA	GTA	TGC	CGG
[441]D	G	L	C	C	D	Q	C	K	F	M	K	E	G	T	V	C	R	[458]
	1469		1478		1487		1496		1505		1514							
	AGA	GCA	AGG	GGT	GAT	GAC	CTG	GAT	GAT	TAC	TGC	AAT	GGC	ATA	TCT	GCT	GGC	TGT
[459]R	A	R	G	D	D	L	D	D	Y	C	N	G	I	S	A	G	C	[476]
	1523		1532		1541		1550		1559		1568							
	CCC	AGA	AAT	CCC	TTC	CAT	GCC	TAA	CCA	ACA	ATG	GAG	ATG	GAA	TGG	TCT	GCA	GCA
[477]P	R	N	P	F	H	A	*	[483]										
	1577		1586		1595		1604		1613		1622							
	ACA	GGC	AGT	GTG	TTG	ATC	TGA	ATA	CAG	CCT	AAT	AAT	CAA	CCT	CTG	GCT	TCT	CTC
	1631		1640		1649		1658		1667		1676							
	AGA	TTT	GAT	CAT	GGA	GAT	CCT	TCT	TCC	AGA	AGG	TTT	CAC	TTC	CCT	CAA	ATC	CAA
	1685		1694		1703		1712		1721		1730							
	AGA	GAC	CCA	TCT	GCC	TGC	ATC	CTA	CTA	GTA	AAT	CAC	CCT	TAG	CTT	CCA	GAT	GGT
	1739		1748		1757		1766		1775		1784							
	ATC	CAA	ATT	CTG	TAA	TAT	TTC	TTC	TCC	ATA	TTT	AAT	CTA	TTT	ACC	TTT	TGC	TGT
	1793		1802		1811		1820		1829		1838							
	AAC	AAA	ACC	TTT	TTC	CTG	TCA	CAA	AGC	TCC	ATG	GGC	ATG	TAC	AGC	TTA	TCT	GCT
	1847		1856		1865		1874		1883		1892							
	GTC	AAG	AAA	AAA	AAT	GGC	CAT	TTT	ACC	GTT	TGC	CAG	TTA	CAA	AGC	ACA	TTT	AAT
	1901		1910		1919		1928		1937		1946							
	GCA	ACA	AGT	TCT	TCC	TTT	TGA	GCT	GAT	GTA	TTC	AAA	GTC	AAT	GCT	TCC	TCT	CCC

FIG. 3D

	1955		1964		1973		1982		1991		2000						
AAA	ATT	TCA	TGC	TGG	CTT	CCC	AAG	ATG	TAG	CTG	CTT	CCG	<u>TCA</u>	<u>ATA</u>	<u>AAC</u>	AAA	CTA
	2009				2018				2027								
TTC	TCA	TTC	<u>AAA</u>	<u>AAA</u>	<u>AAA</u>	<u>AAC</u>	CCG	AAT	TC	3'							

FIG. 4A

Proprotein domain:

	1	10	20	30	40	50
	*	*	*	*	*	*
CN	MIQVLLVTLCIAAFPYQGSSIIILESGNVNDYEVLYPQKVLTALPKGAVQPKY					
Trigramin	MIQVLLITICLAVFPYQGSSIIILESGNLNDYEVVYPEKVTALPKGAVQPKY					
Cat	MIQVLLVTICLAAFPYQGSSIIILESGNVNDYEVYIPRKVTALPKGAVQPKY					
Jararhagin						ATRPKGAVQPKY
Ht-e	MIQVLLVTICLAAFPYQGSSIIILESGNVNDYEVYIPRKVTALPKGAVQPKY					
		110	120	130	140	150
		*	*	*	*	*
CN	DHCYYHGRIQNDADSTASISACNGLKGHFKLQGETYLIIEPLKLSDEAHAV					
Trigramin	DHCYYHGRIENDADSTASISACDGLKGHFKLQGEMYLIEPLELSDSEAHAV					
Cat	DHCYYHGRIENDADSTASISACNGLKGHFKLQGEMYLIEPLKLPDSEAHAV					
Jararhagin	DHCYYHGRIENDADSTASISACNGLKGYFKLQRETYFIEPLKLPDSEAHAV					
Ht-e	DHCYYHGRIENDADSTASISACNGLKGHFKLQGEMYLIEPLKLSDEAHAV					

Metalloproteinase domain:

	200	210	220	230	240
	*	*	*	*	*
CN	EQQGF.PQRYIELVVVADHRMFTKYNGNLNTIRIWWHEL VNTMNVFYRPLN				
Trigramin	EQQRF.PQRYIKLGIFVDHGM YTKYSGNSERITKRVHQM INNINMMCRALN				
Cat	EHQKYNPFRFVELFLVVDKAMVTKNNGDLDKIKTRMYEIVNTVNEIYRYMY				
Jararhagin	EQQRYDPYKYIEFFVVVDQGT VTKNNGDLDKIKARMYELANIVNEIFRYLY				
Ht-e	EHQ.....RYVELFIVVDHGM YTKYNGSDKIRQRVHQM VNIMKESYTYMY				
	290	300	310	320	330
	*	*	*	*	*
CN	LTAIELDGETIGLANRGTMCDPKLSTGIVQDHSAINLWVAVTMAHEMGNL				
Trigramin	LTATIFNGNVIGRAPVGGMCDPKRSVAIVRDHNAIVFVAVTMTHEMGNL				
Cat	LTAIDL.DRVIGLAYVVGSMCHPKRSTGIIQDYSEINLVVAVIMAHEMGNL				
Jarahagin	LTAIDFNGPTIGYAYIGSMCHPKRSVGIVQDYSPINLVVAVIMAHEMGNL				
Ht-e	LTSIAFDEQIIGRAYIGGICDPKRSTGVVQDHSEINLRVAVTMTHELGNL				

Disintegrin domain:

	420	430	440	450
	*	*	*	*
CN	ETGEESDF---DAOABOCCDAATCJKTTSQCADGKCCDQCJFNJEGTVCR			
Trigramin	EAGEDCDGSPA...NPCCDAATCKLIPGAQCGEGLCCDQCSFIEEGTVCR			
Cat	EVGEECDGTPENCQNECCDAATCKLKSGSQCGHGDCEQCKFSKSGTECR			
Jararhagin	EVGEECDGTPENCQNECCDAATCKLKSGSQCGHGDCEQCKFSKSGTECR			
Ht-e	EAGIECDGGSLE...NPCCYATTCKMRPGSQCAEGLCCDQCRFMKGTVC			

C-terminal domain:

	490	500	510	520	530
	*	*	*	*	*
Cat	NGQPCLDNYGYCYNGNCPIMYHQC YDLFGADVYEAEDSCFERNQKGNYGY				
Jararhagin	NGQPCLDNYGYCYNGNCPIMYHQC YALFGADVYEAEDSCFKDNQKGNYGY				
	590	600			
	*	*			
Cat	PGTKCADGKVC SNHGCV DVATAY*				
Jararhagin	PGTKCADGKVC SNHGCV DVATAY				

FIG. 4B

60	70	80	90	100	
*	*	*	*	*	
EDTMQYEFKVNGEPPVVLHLEKNKGLFSKDYSETHYSSDGRKITTNPVE					
EDAMQYEFKVNGEPPVVLHLEKNKGLFSEDYSEIHYS PDGREITAYPSVE					
EDAMQYELKVNGEPPVVLHLGKNKGLFSKDYSETHYS PDGREITTYPLVE					
EDAMQYEFKVNGEPPVVLHLEKNKGLFSKDYSEIHYS PDGREITTYPPVE					
EDTMQYELKVNGEPPVVLHLEKNKGLFSKDYSETHYSFDGRKITTNP SVE					
160	170	180	190		
*	*	*	*		
YKYENVEKEDEAPKMGVTQTNWESDEPIKKASQLNLTP					
FKYENVEKEDEPPKMGVTQ.NWESYESTKKASQLNVTP					
YKYENVEKEDEALKMGVTQ.NWESYEPIKKASQLVVT A					
FKYENVEKEDEAPKMGVTQ.NWKSYEPIKKASQLAFTA					
FKLKNVEKEDEAPKMGVTQ.NWESYEPIKKASDLNLNP					
250	260	270	280		
*	*	*	*		
IRVSLTDLEVWSDQDLINVQPAAADTLEAFGD.WRET VLLNRISHDNAQL					
IVTTLSVLEIWSEKDLITVQ.ASAPTTLTLFGAWRET VLLNRTSHDHAQL					
IHVALVGLEIWSNEDKITVKPEAGYTLNA.FGEWRKTDLLTRKKHDNAQL					
MHVALVGLEIWSNGDKITVKPDVDYTLNS.FAEWRKTDLLTRKKHDNAQL					
IDILLAGIEIWSNGDLINVQPASPNTLNS.FGEWRET DLLKRKSHDNAQL					
350	360	370	380	390	400 410
*	*	*	*	*	* *
<u>G</u> ISHDGNQCHCDANSCIMSEELREQLSFEFSDCSQNYQTYLTDHNPQCMLNEPLRTDIVSTPVSGNELL					
<u>G</u> MHHDEDKCNCN..TCIMSKVLSRQPSKYFSECSKDYYQTFLT NHNPQCILNAPLRTDTVSTPVSGNELL					
<u>G</u> INHDSGYCSCGDYACIMRPEISPEPSTFFSNCSYFECWDFIMNHNPECILNEPLGTDII SPPVCGNELL					
<u>G</u> IHHDTGSCSCGDYPCIMGPTISNEPSKFFSNCSYIQCWDFIMNHNPECIINEPLGTDII SPPVCGNELL					
<u>G</u> IHHDTDSCSCGGYSCIMSPVISDEPSKYFSDCSYIQWFEFIMNQKPQCILKKPLRTDTVSTPVSGNELL					
460	470	480			
*	*	*			
<u>R</u> ARGD.DLDDYCNGISAGCPRNPFHA*					
<u>I</u> ARGD.DLDDYCNGRSAGCPRNPFHA					
ASMSECDPAEHCTGQSSECPADV FHK					
ASMSECDPAEHCTGQSSECPADV FHK					
VSMVDRN.DDTCTGQSADCPRNGLYG*					
540	550	560	570	580	
*	*	*	*	*	
CRKENG NKIPCAPEDVKCGRLYCKDN SPGQNNPCKMFYSNEDEHKGMVL					
CRKENG KKI PCAPEDVKCGRLYCKDN SPGQNNPCKMFYSNDDEHKGMVL					

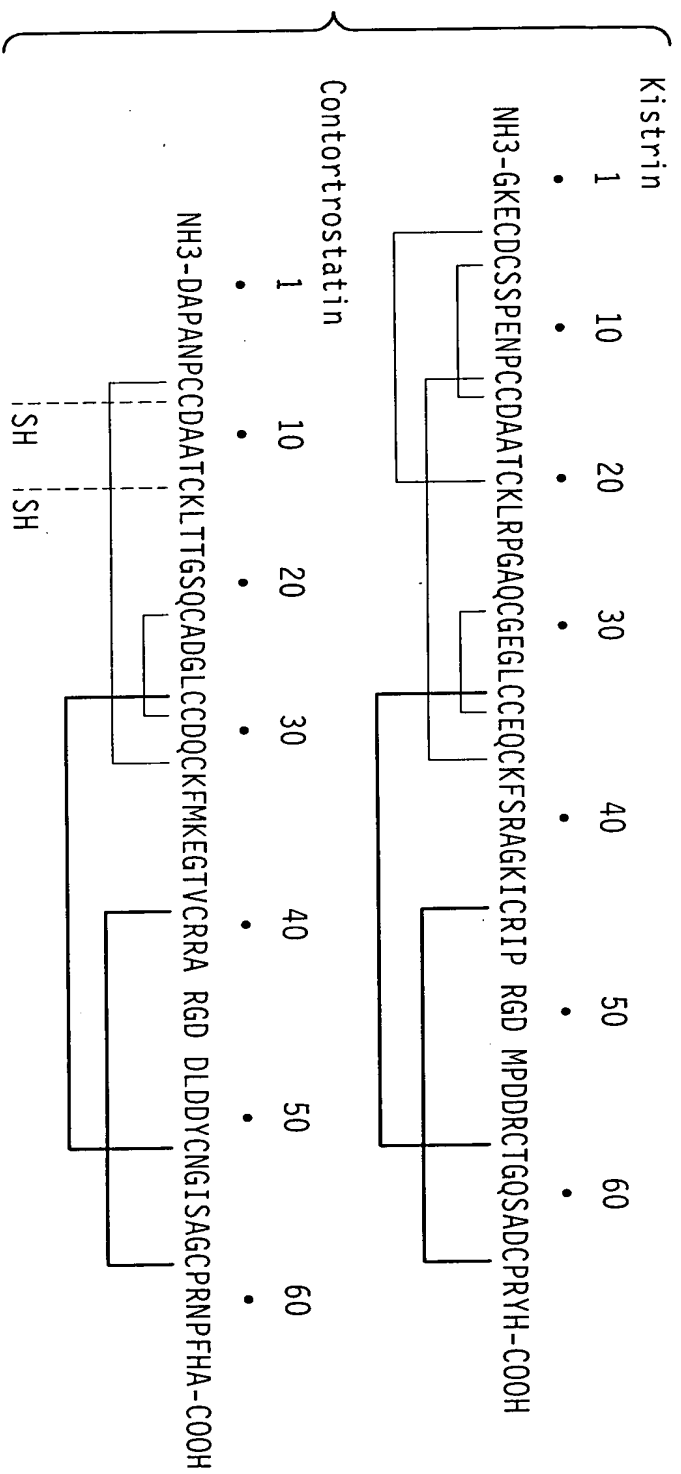


FIG. 5A

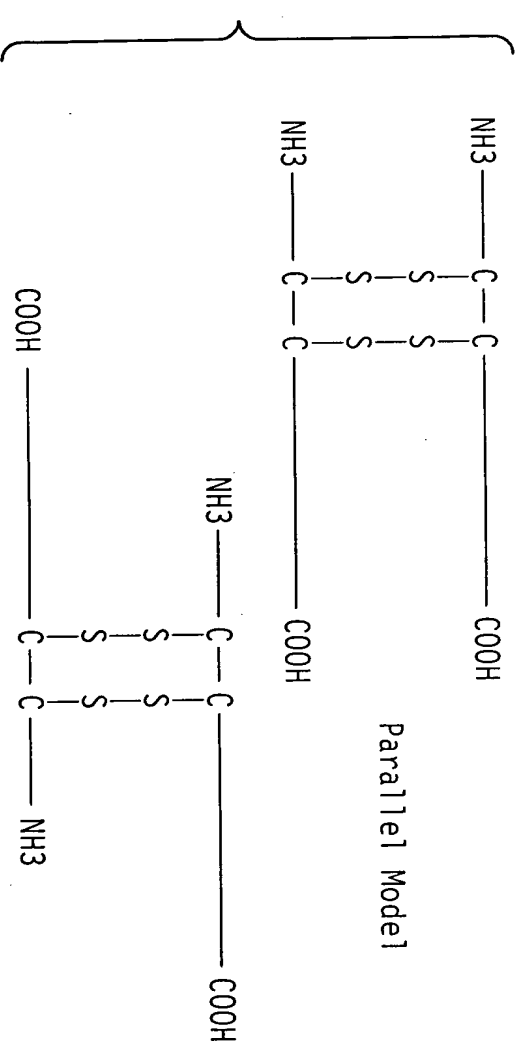


FIG. 5B

FIG. 6

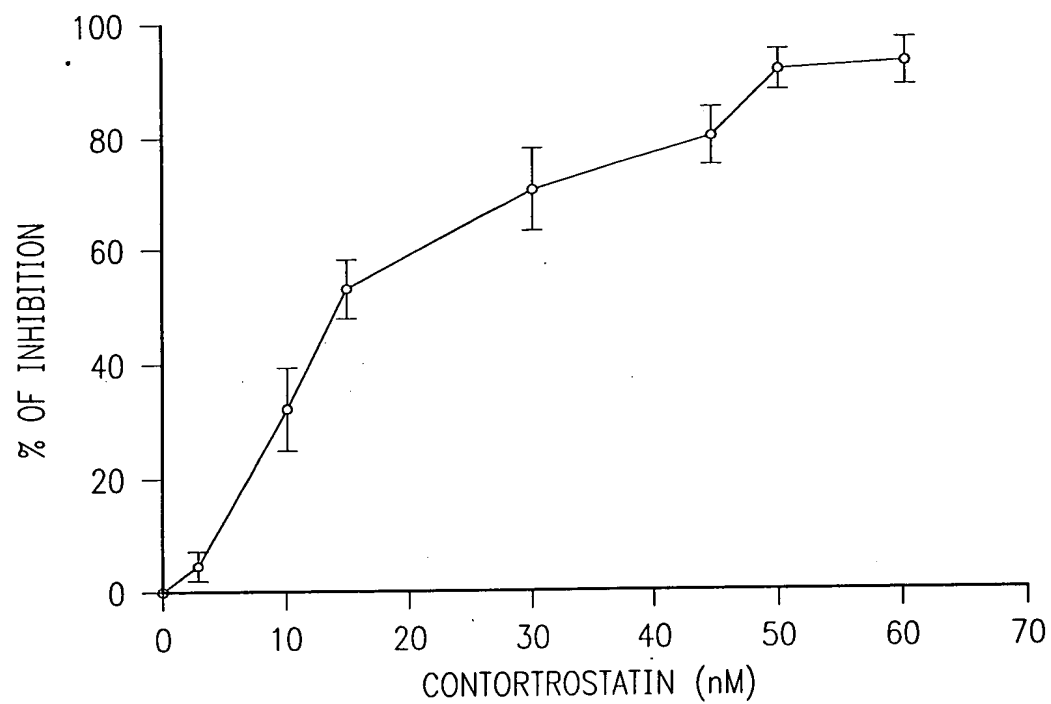


FIG. 7

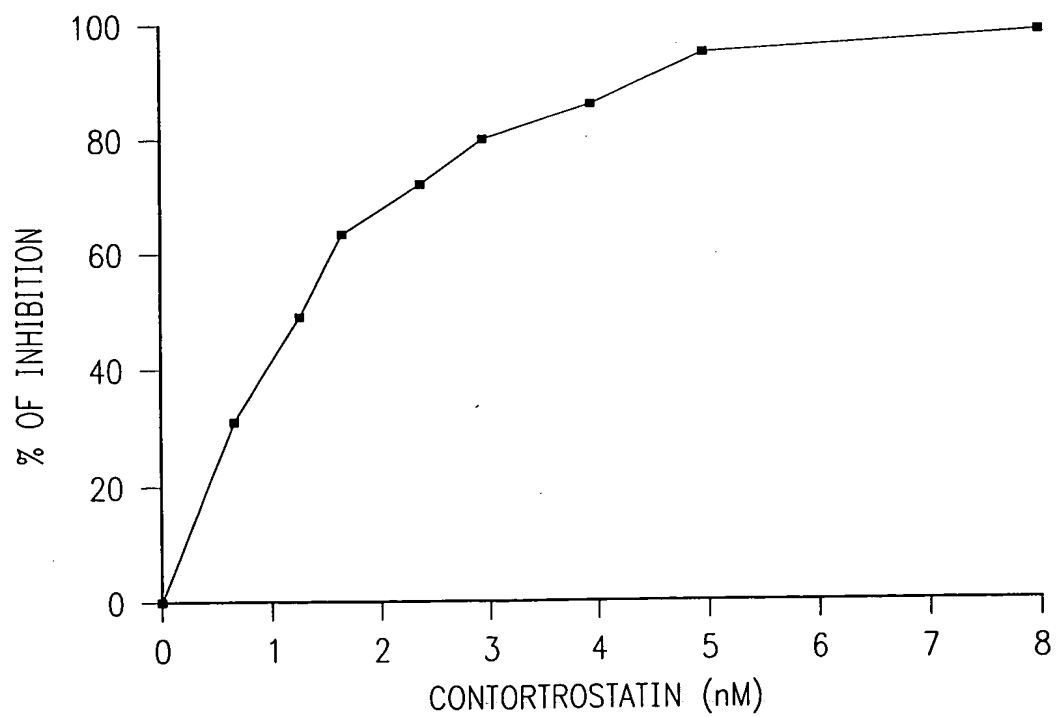


FIG. 8

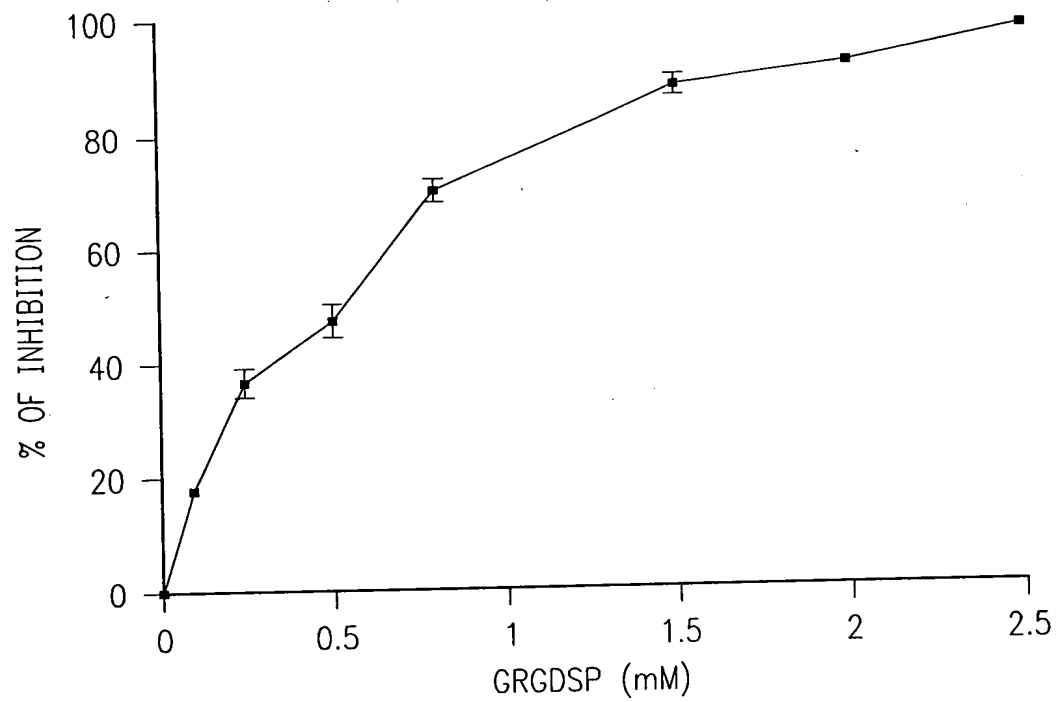
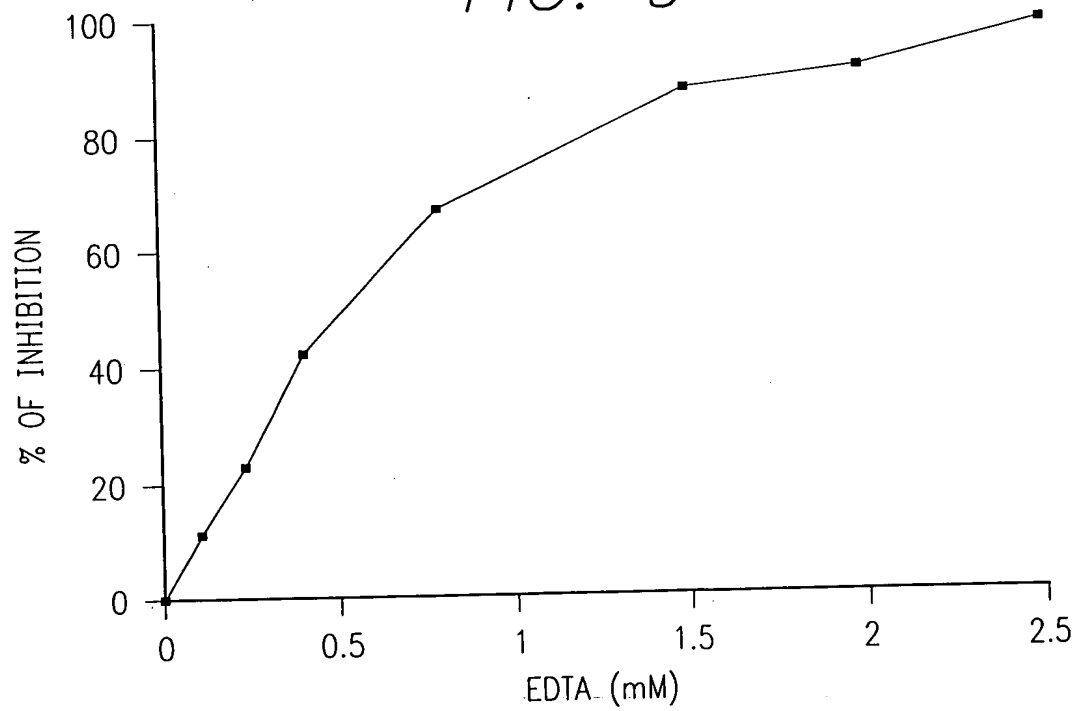


FIG. 9



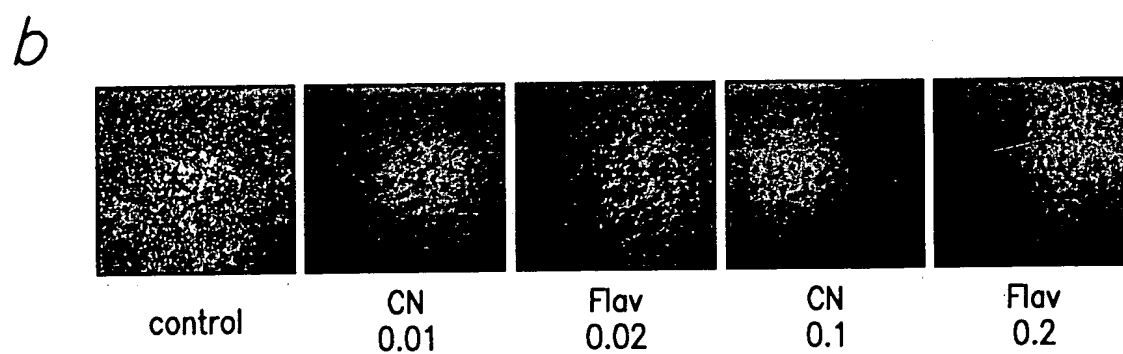
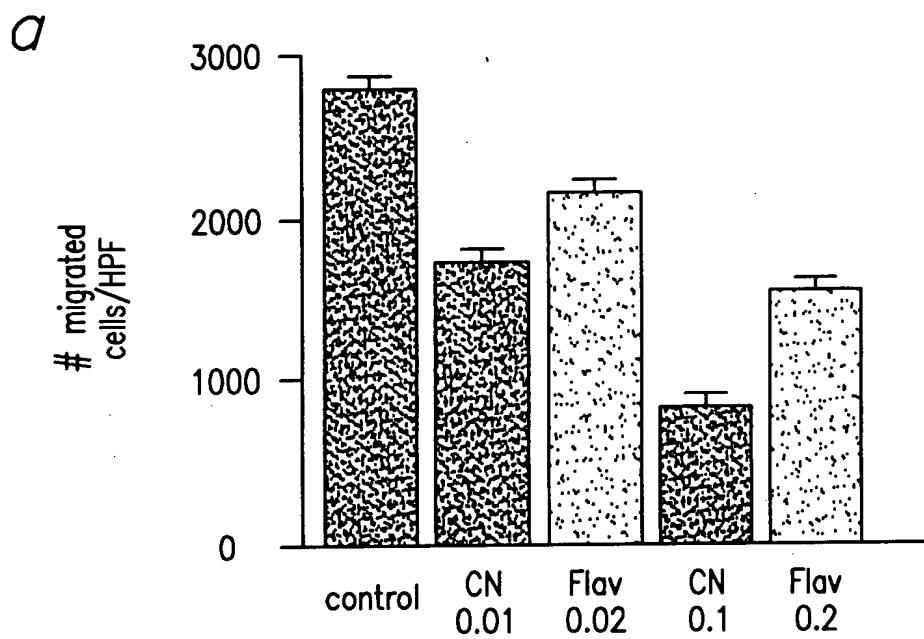


FIG. 10

FIG. 11

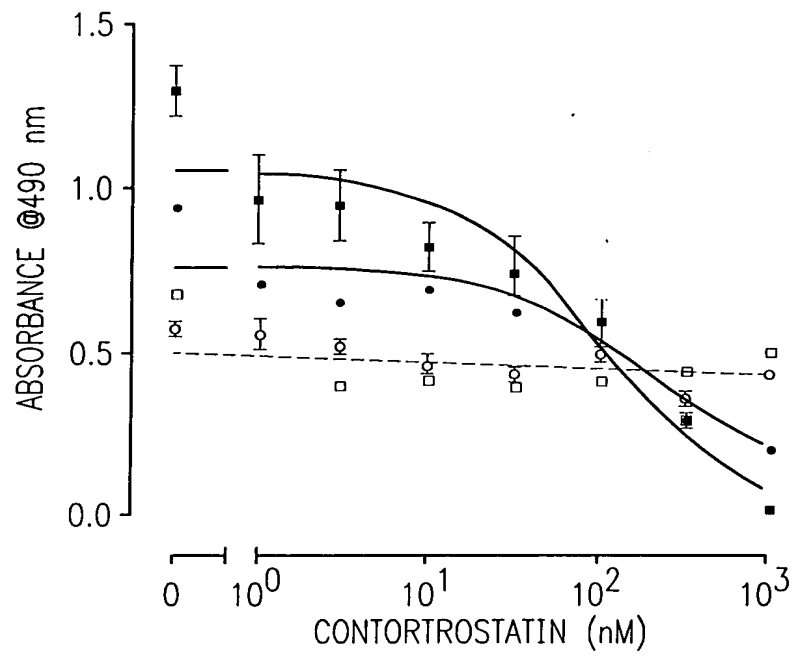
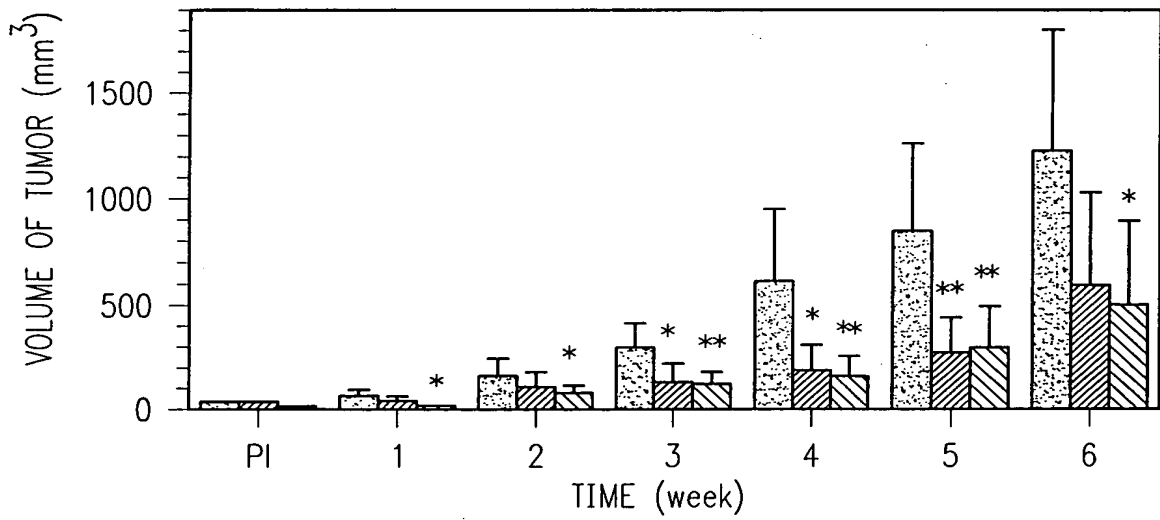


FIG. 12



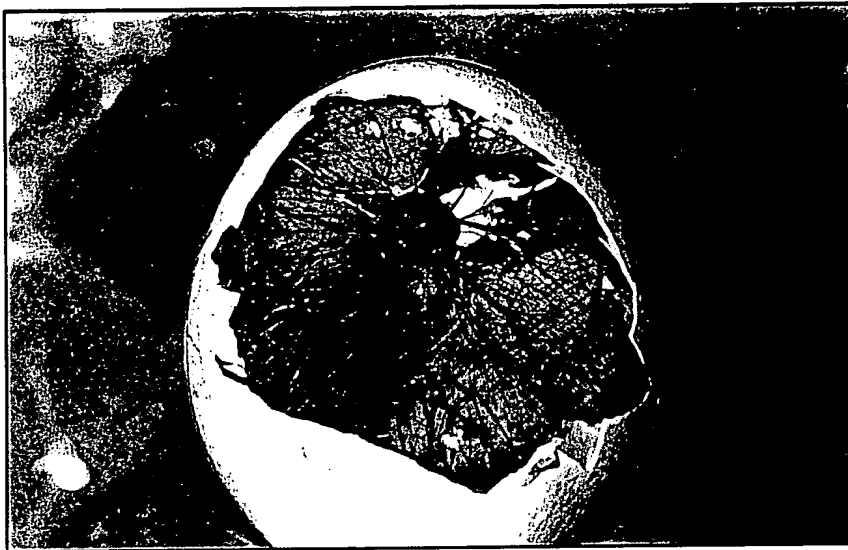


FIG. 13A

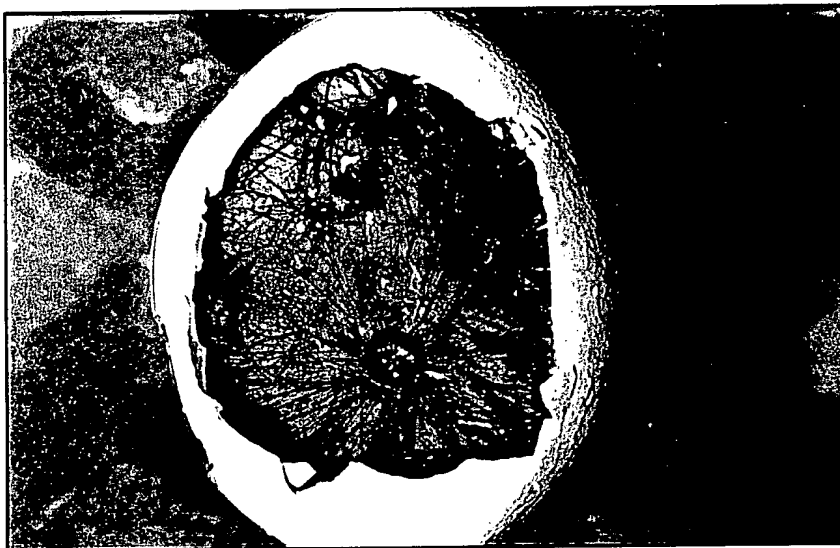
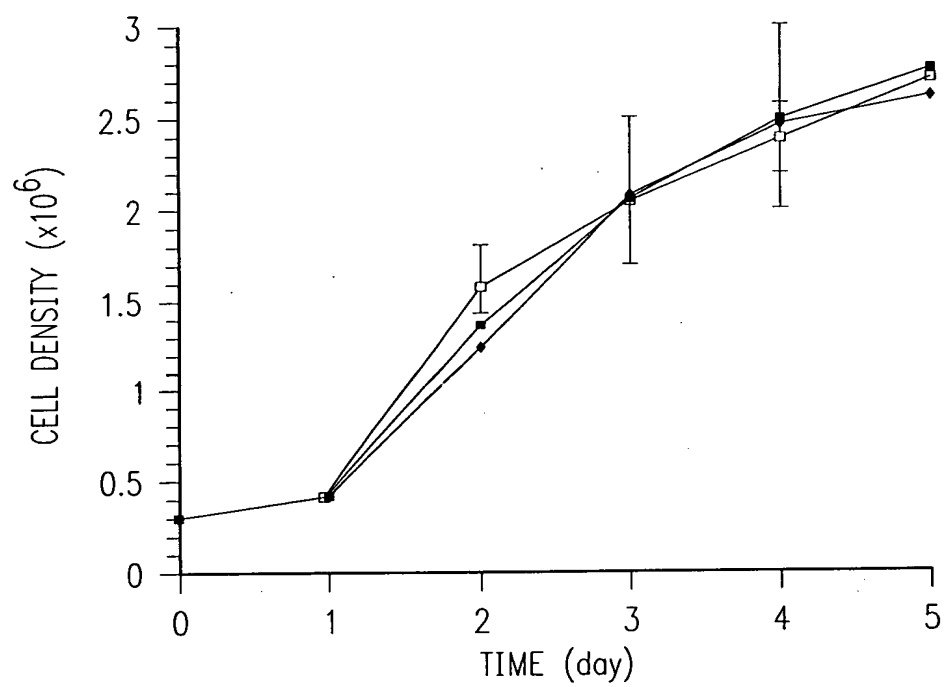


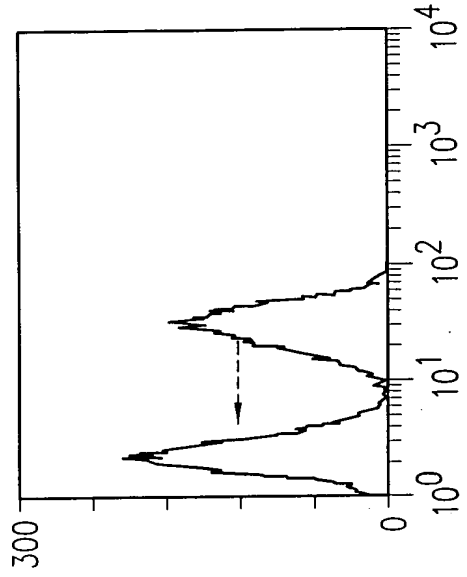
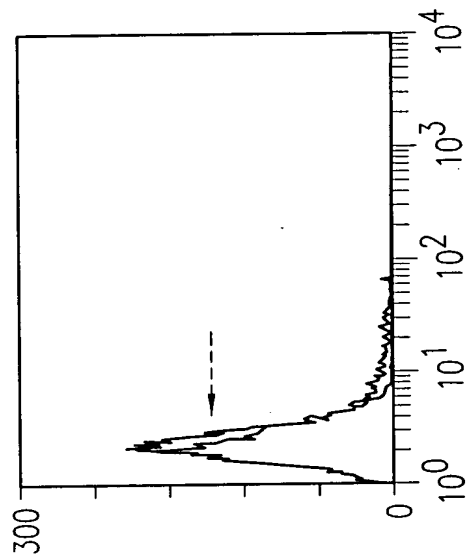
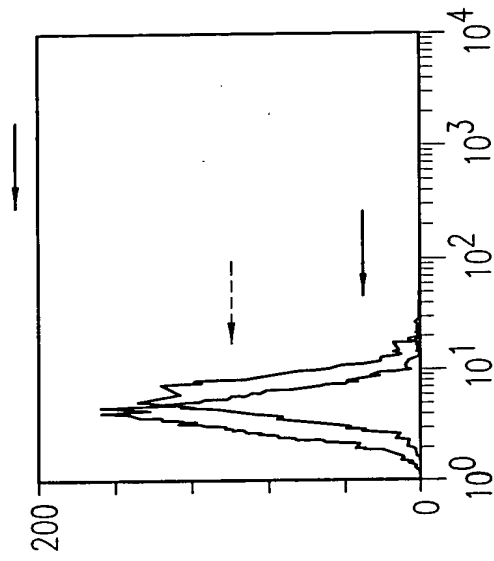
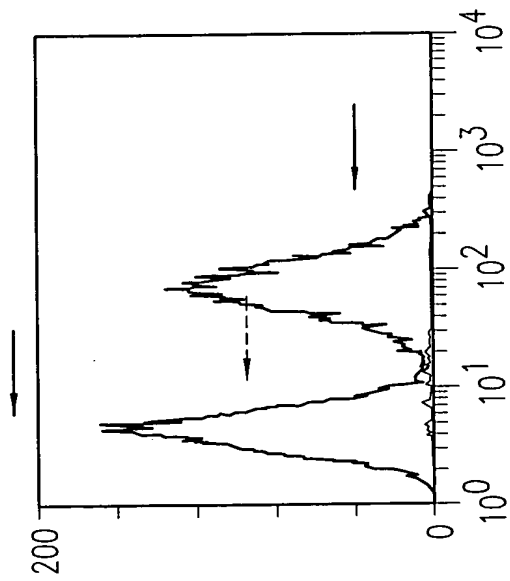
FIG. 13B



FIG. 13C

FIG. 14





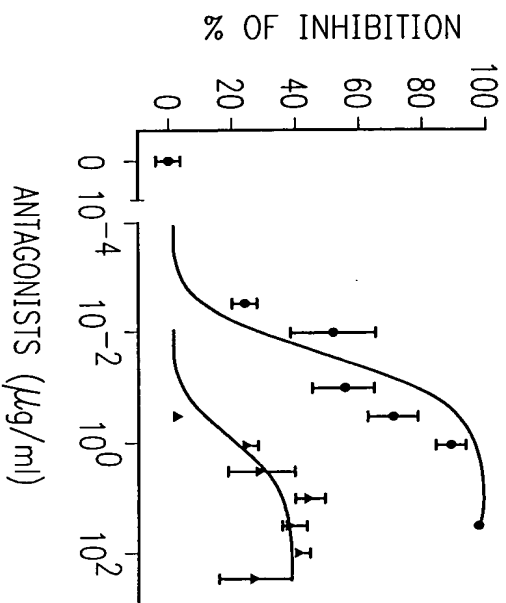


FIG. 16A

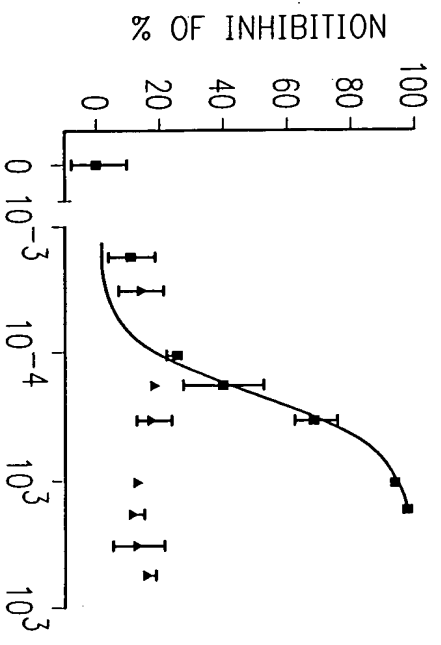


FIG. 16C

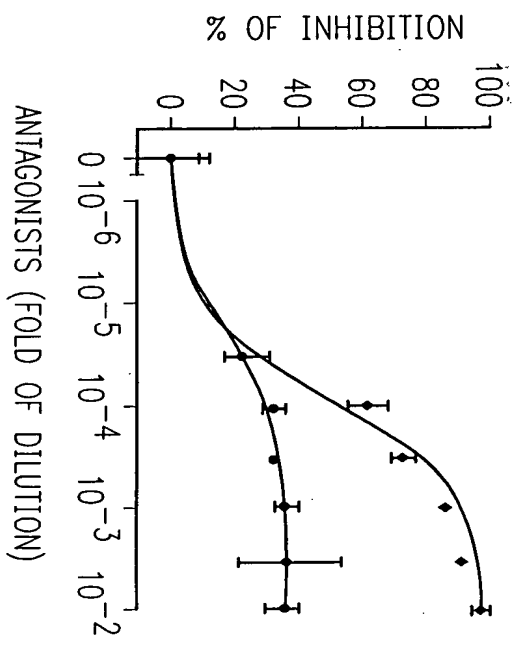


FIG. 16B

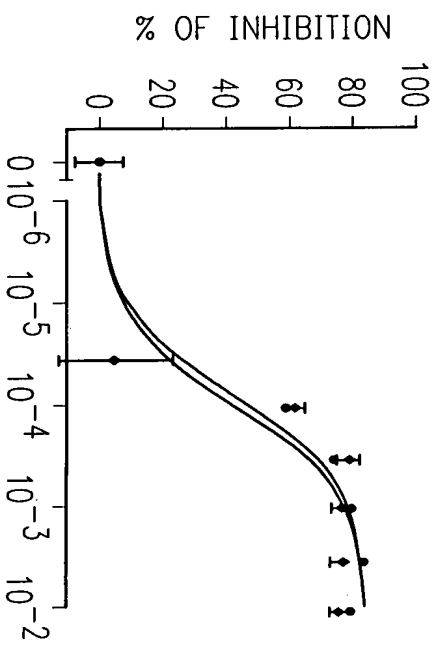


FIG. 16D

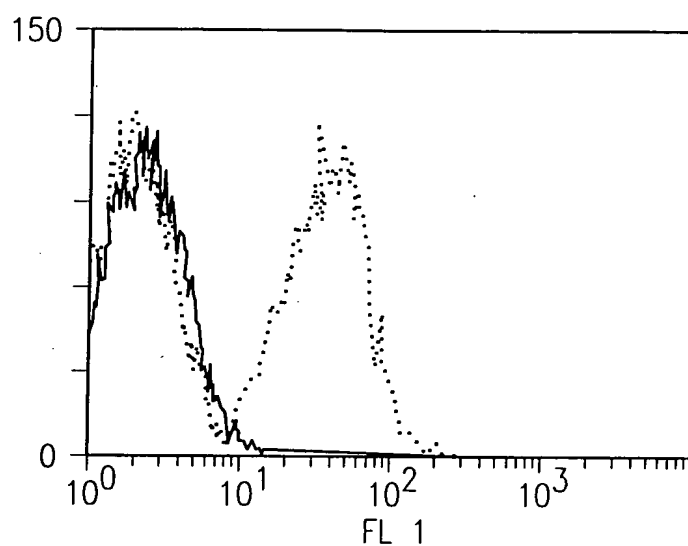


FIG. 17

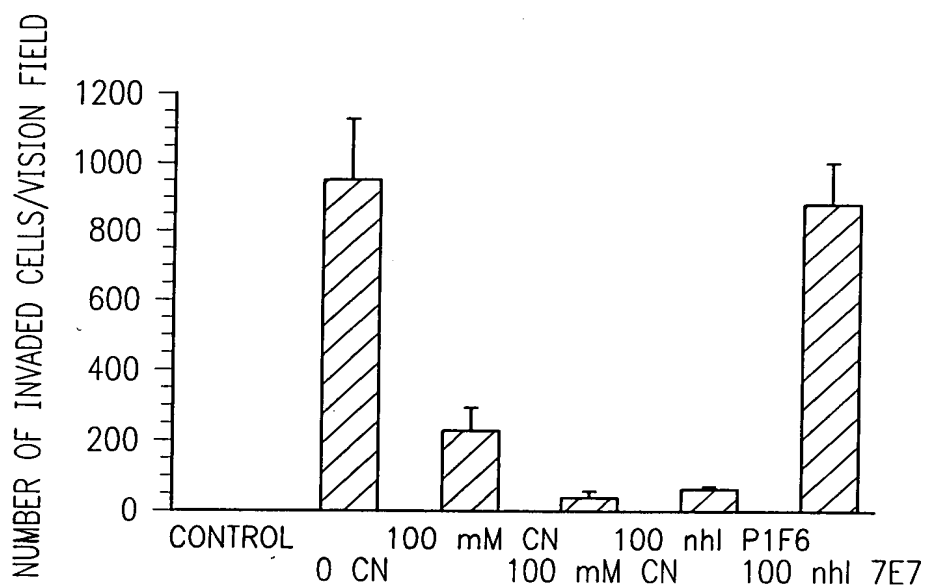


FIG. 18

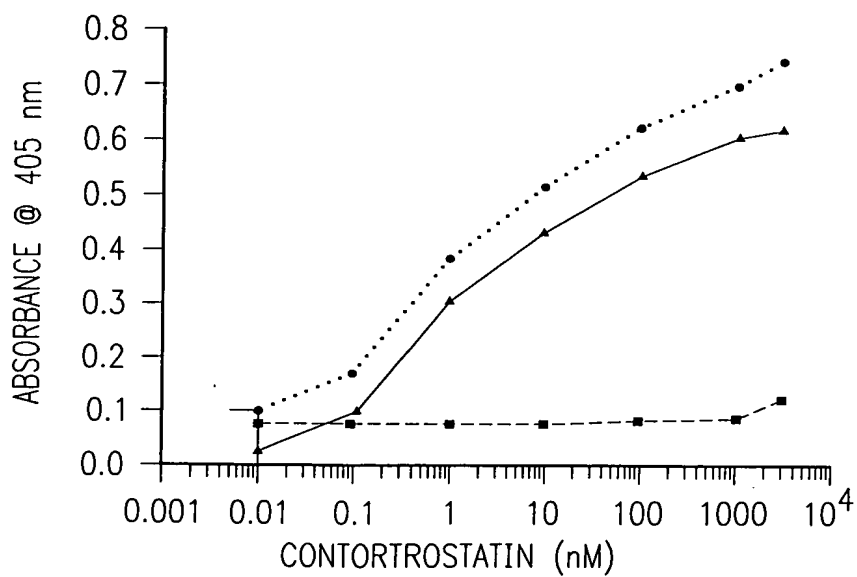


FIG. 19

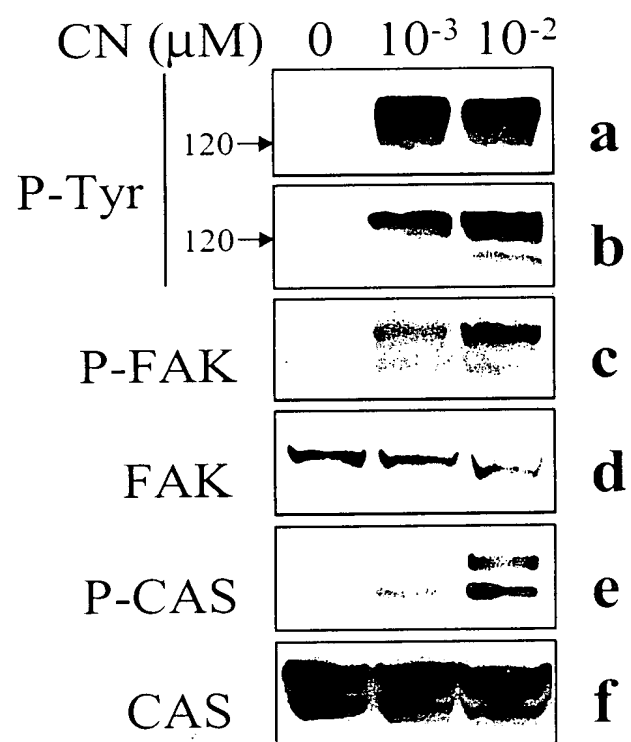


FIG. 20

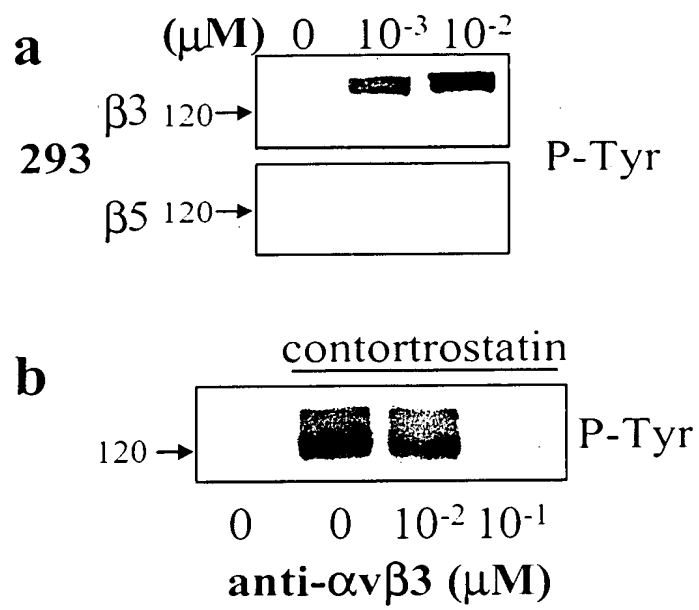


FIG. 21

FIG. 22

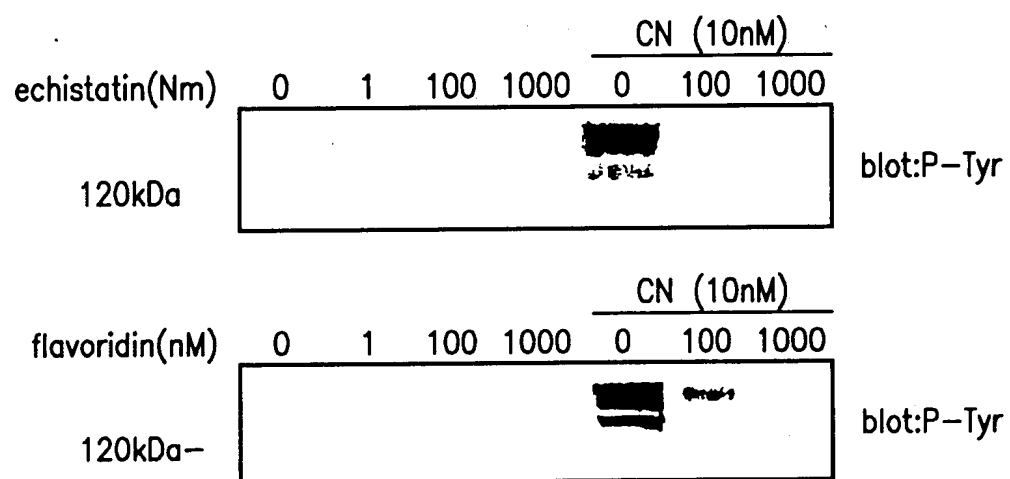


FIG. 23

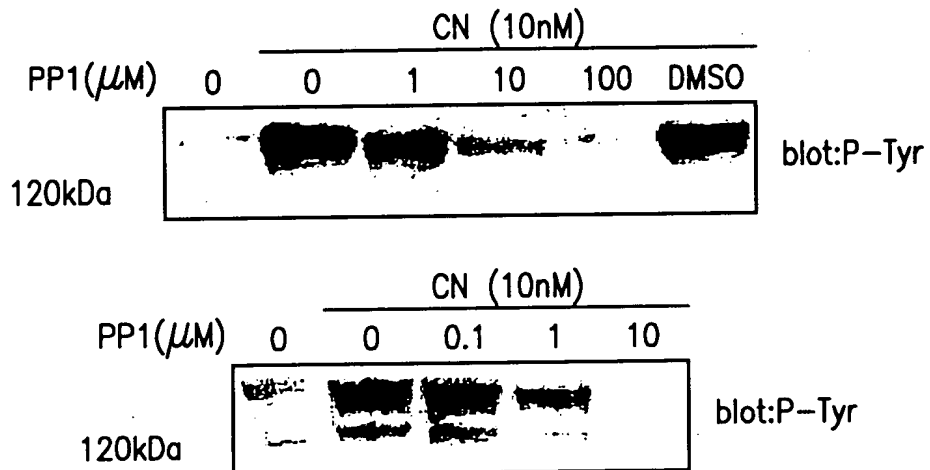
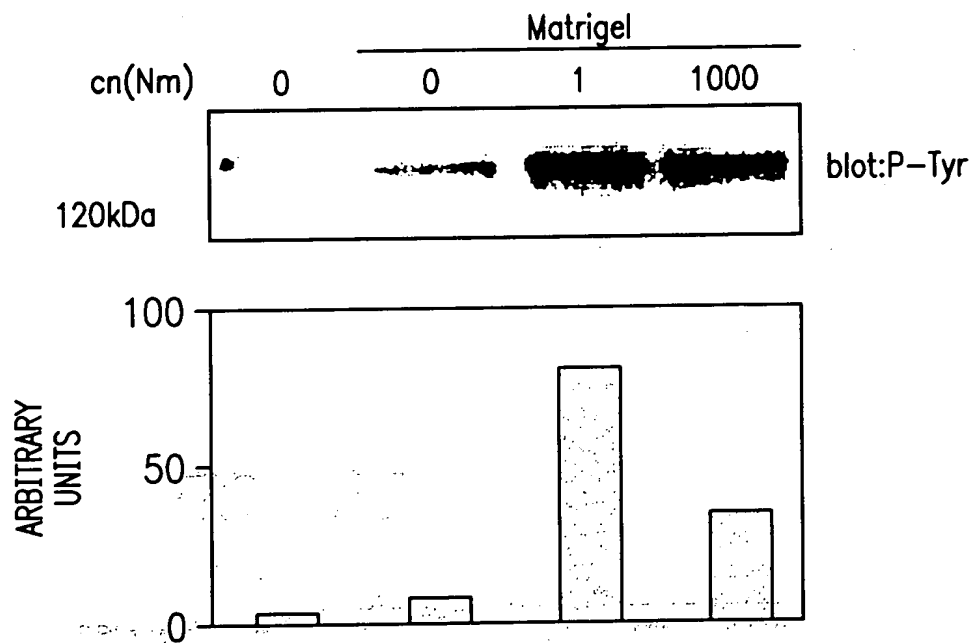


FIG. 24



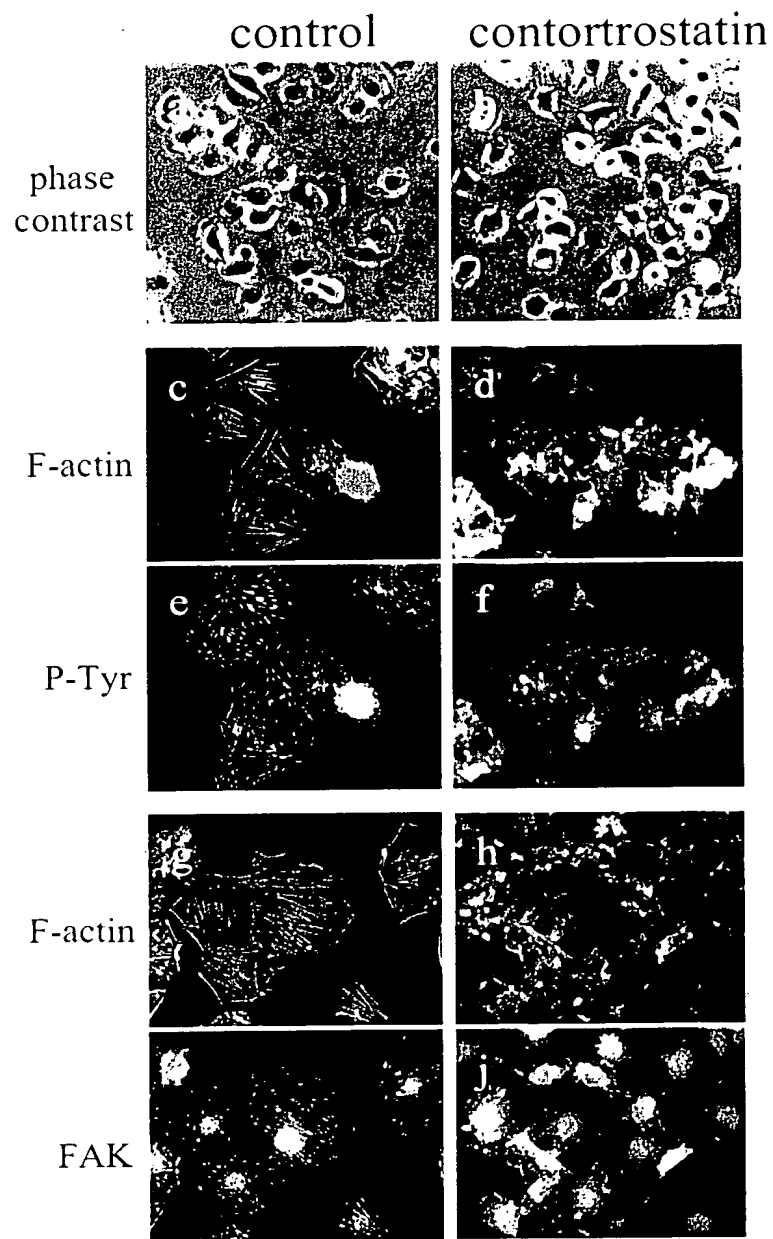


FIG. 25

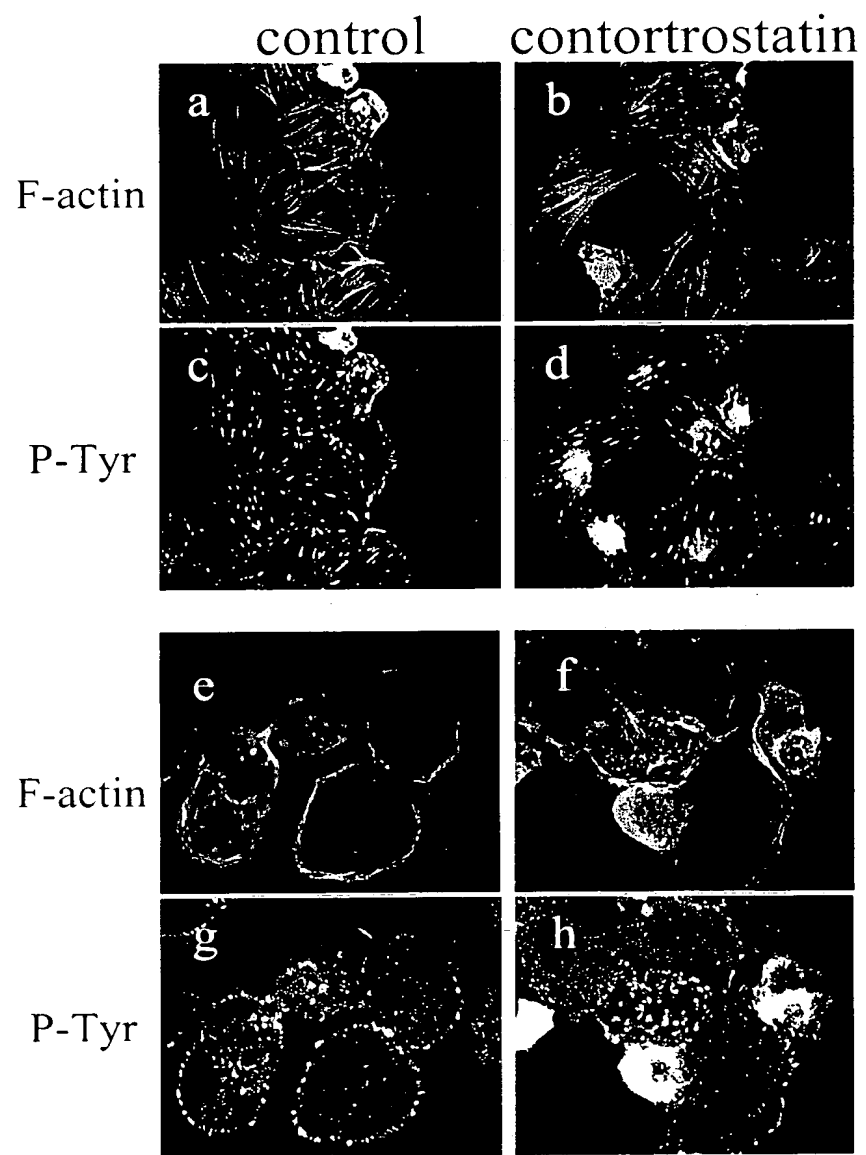


FIG. 26

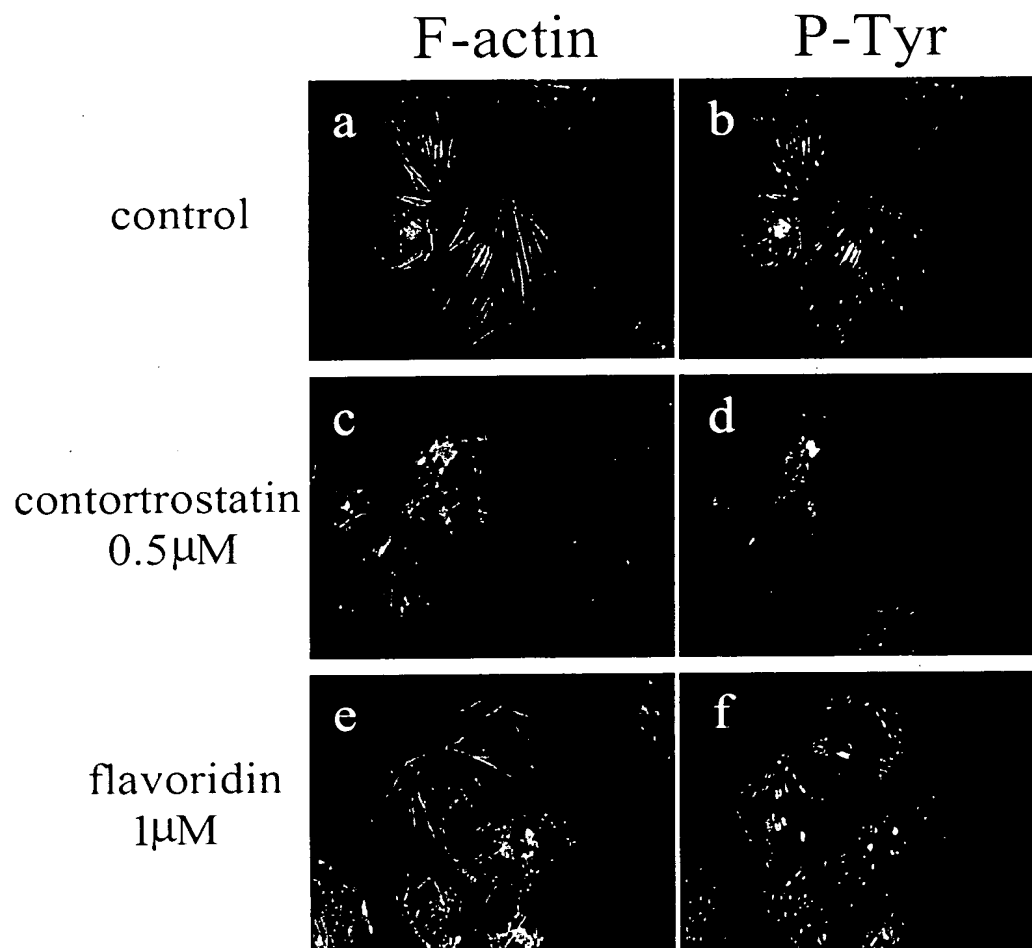


FIG. 27

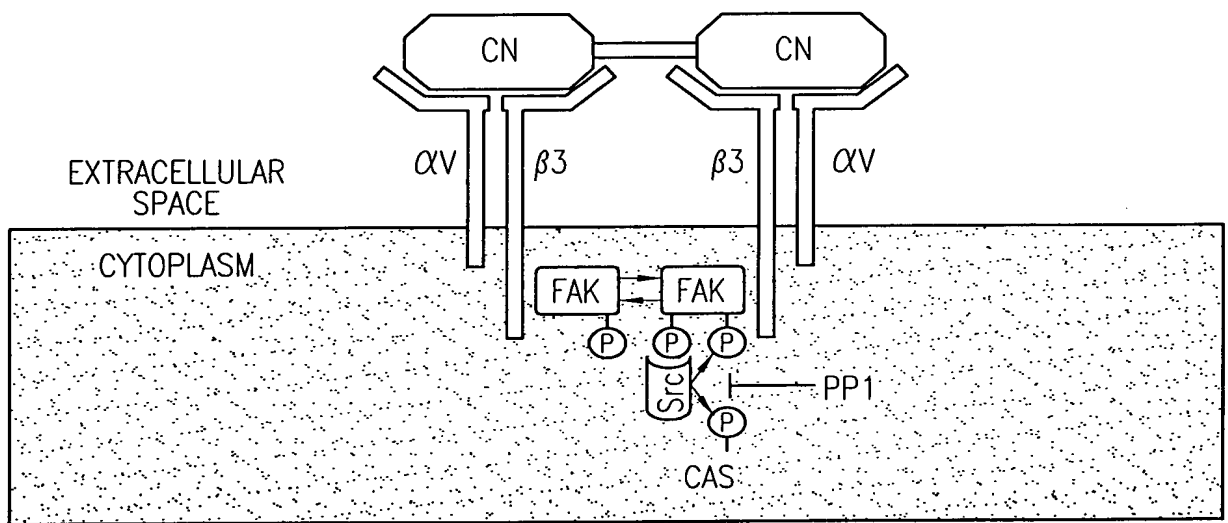


FIG. 28

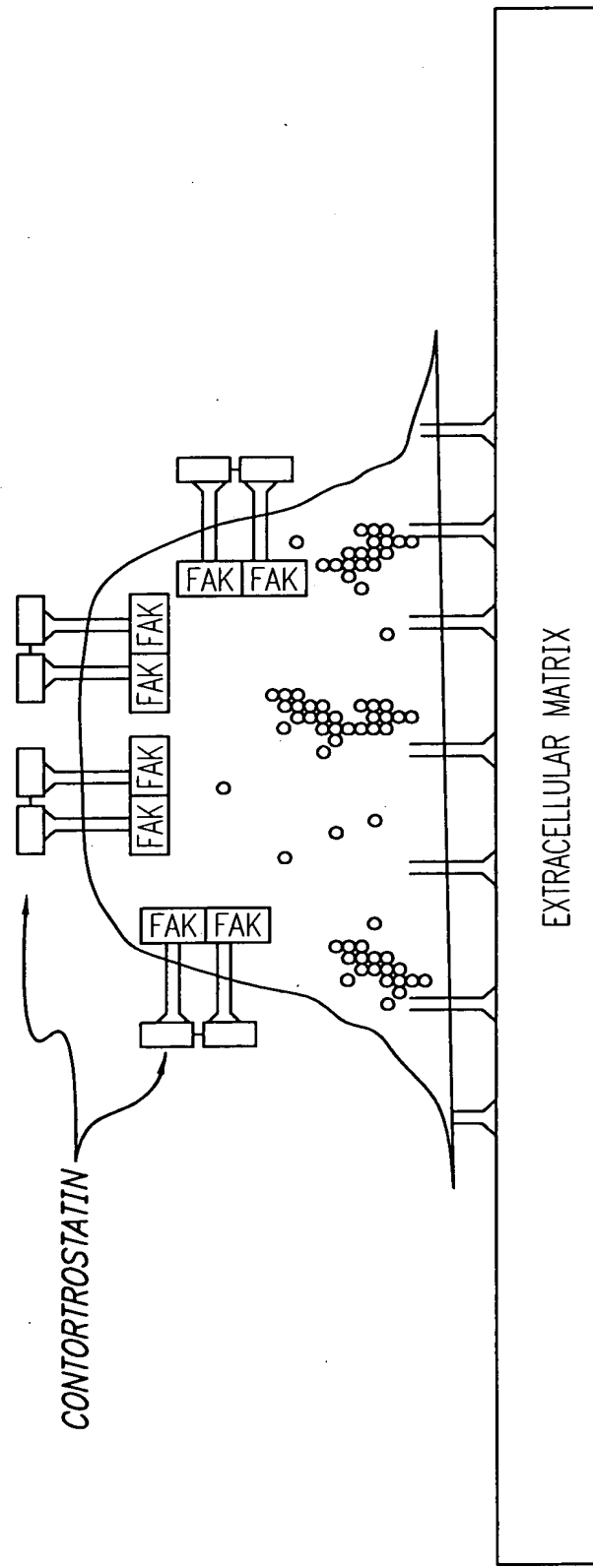
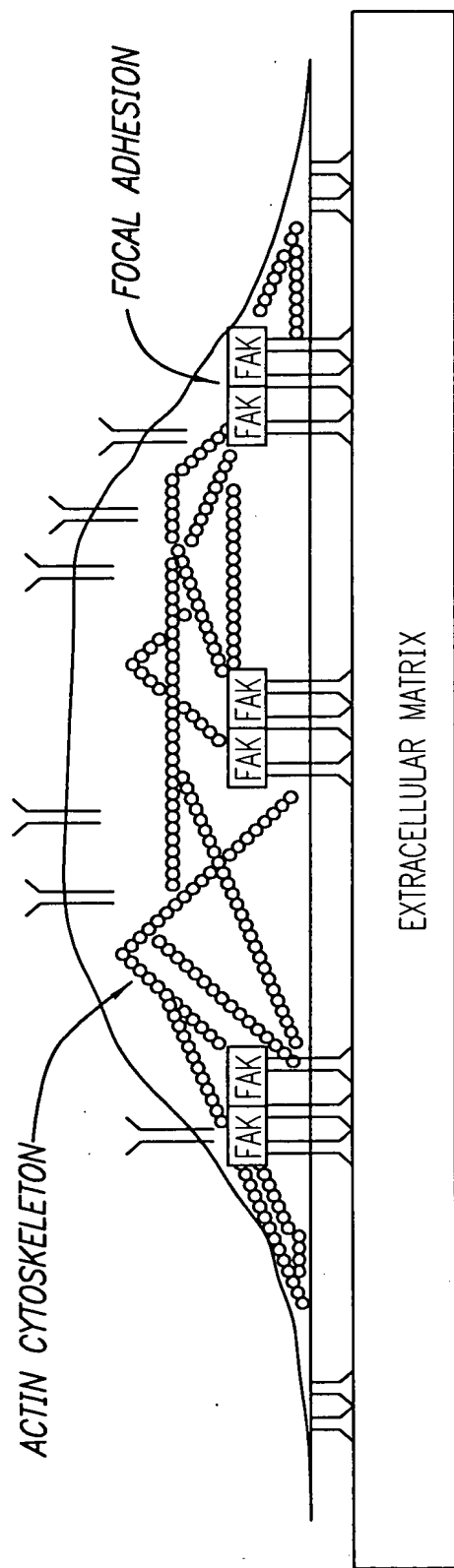


FIG. 29